

Molecular Identification of *Caulerpa selago* as a New Record For The Persian Gulf

Mojib Salehi balashahri¹, Jelveh Sohrabipour², Mohammad Mehdi Dehshiri^{3*}, Iraj Mehregan⁴

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Abstract

Caulerpa is a genus of siphonous green algae belonging to Caulerpaceae which consists of about 103 accepted species of tropical to subtropical seas. Recently, some species of the genus have expanded their distribution into more temperate environments such as the Mediterranean Sea. Due to their bioactive compound and secondary metabolites, content species of this genus have many medicinal uses in various countries, especially in some countries such as the Philippines, Malaysia, and Vietnam, which are used as vegetables. The extract of this alga has antibacterial, anti-viral, and anti-inflammatory properties. Due to this economic and scientific importance, accurate identification of these species is very important. Morphological similarities between some species of this genus and phenotypic changes caused by ecological factors have caused some complications and problems with inaccurate identification at the species level. For this reason, in addition to morphological features, phyloge-

netic analyzes are also used to accurately identification of the members of this genus. In this study, a molecular phylogeny was inferred from chloroplast *tufA* sequences of 35 taxa, which of them a sequence of *CaulerPELLA ambigua* used as an outgroup. The results confirmed the presence of *Caulerpa selago* in the algal flora of Iran which is the first record of this species from the coastlines of the Persian Gulf and Gulf of Oman in the south of Iran.

Keywords: *Caulerpa selago*, Persian Gulf, Gulf of Oman, *tufA* gene.

Introduction

Caulerpa is a genus of seaweed in Caulerpaceae (green algae). This family belongs to the Bryopsidophyceae (Van den Hoek et al., 1995), a class consisting of algae with the coenocytic thallus. Their thallus is composed essentially of a single cell that develops into a system of branching siphons, stolons, and erect branches. The family Caulerpaceae includes two genera,

1- Islamic Azad University, Science and Research Branch, Tehran, Iran

2- Agriculture and Natural Resources Research and Education center of Hormozgan, Agriculture Research, Education and Extension Organization (AREEO)

3- Department of Biology, Borujerd Branch, Islamic Azad University, Borujerd, Iran

4- Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

*Email address: m.m.dehshiri@iau.ac.ir

Caulerpa J.V. Lamouroux (a species-rich genus) and the monotypic genus *Caulerpella* Prudhomme and Lokhorst (Draisma et al., 2014). Thalli of the genus *Caulerpa* are composed of a horizontal rhizome (stolon), downward branched rhizoids, and upright branches (assimilators) that bear distinctive branchlets (ramuli), which the characteristics are mainly used in species identification (Fritsch, 1965). More than 350 species and infraspecific taxa globally are reported as members of the genus *Caulerpa* (Belton et al., 2014), of which 103 taxa have been verified as accepted taxon (Guiry and Guiry, 2021). Species of the genus are characterized by differences in branching patterns of erect fronds, forms and arrangement of branchlets (ramuli), and stolon forms. These different morphological characters are commonly used for species delimitation conditions which cause phenotypic plasticity in all these characters, also leads to much confusion, and results in a large number of synonyms, including subspecies, varieties, forms, and “ecads” (Coppejans and Prud’homme van Reine, 1992; Belton et al., 2014). This Phenotypic plasticity is the source of taxonomic difficulty in many algal groups, sometimes leading to misidentifications that cause incorrect results in biodiversity estimation and confusing classification (Belton et al., 2014). Molecular study as a modern tool helps phycologists delimit this problem and achieve a convenient category in taxonomically challenging groups (Saunders and Kucera, 2010; Belton et al.,

2014). Some molecular studies (Sauvage et al., 2013; Belton et al., 2014) showed the need for taxonomic revision in the *Caulerpa* genus.

Some *Caulerpa* species, such as *Caulerpa cylindracea* and *C. taxifolia*, may cause severe environmental damage as invasive species (Preez et al., 2020). *C. lentillifera* (sea grapes) commercially has grown in ponds and consumed in the Philippines, Indonesia, and Vietnam. The species is rich in polyunsaturated fatty acids (PUFAs) (Saito et al., 2010), multiple essential amino acids, minerals, dietary fibers, vitamin A and Vitamin C (Matanjun et al., 2009), and has low levels of lipids (Niwano et al., 2009). The species can have improved metabolism of glucose, reduced inflammation, decreased bodyweight, reduced LDL, and increased HDL-cholesterol concentrations in high-fat diet-fed rats (Matanjun et al., 2009, 2010). In addition, *Caulerpa* species have some secondary metabolites such as flavonoids, Caulerpin, caulerpenyne (Meyer and Paul, 1992), and siphonaxanthin, which is a carotenoid found in green algae (Zheng et al., 2018). The antidiabetic effect of *C. lentillifera* has also been shown in a study by Khairuddin et al. (2020). Some species of this genus, such as *C. racemosa* used in manufacturing lotions due to the high level of antioxidants compounds that are good for the skin (Susilowati et al., 2019).

Diini (2013) has stated that one marine resource that has the potency to develop as functional foods is *C. racemosa*.

Some species can be aggressive from time

to time, such as *C. taxifolia* and *C. cylindracea*, which cause many problems in the Mediterranean Sea (Najdek et al., 2020a; 2020b; Chen et al., 2019). Due to this economic and scientific importance, accurate identification of the members of this genus is essential.

For the first time, a species of *Caulerpa*, *C. sertularioides*, and one new variety, *C. sertularioides* var. *farlowii*, were reported from the Persian Gulf (Nizamuddin and Gessner, 1970). Sohrabipour and Rabiei (1996) reported 16 new records of marine algae, of which seven species, including *C. peltata* and *C. racemosa* were the new records for the Persian Gulf. Also, Sohrabipour and Rabiei (1999a, b) published a list of seaweeds from the shores of the Persian Gulf and the Gulf of Oman, in which 153 species of algae (including seven species of *Caulerpa*) were reported. In 2007, Sohrabipour and Rabiei presented a checklist of 62 species of green seaweeds from Iranian seashores of the Persian Gulf and Gulf of Oman. This checklist reported 10 species of the genus *Caulerpa*, including *C. fastigiata*, *C. manorensis*, *C. Mexicana*, *C. peltata*, *C. racemosa* var. *macrophysa*, *C. sertularioides* f. *farlowii*, *C. sertularioides* f. *sertularioides*, *C. taxifolia*, *C. brachypus* and *C. scalpelliformis*. Some other studies on marine algae on the Iranian coast of the Persian Gulf and the Gulf of Oman have mentioned some species of *Caulerpa* (Gharanjik, 2000; RohaniGhadekolaei et al., 2007; Shams and GhaedAmini (2017).

Some *Caulerpa* species also have been re-

ported from Arabian countries at southern coastlines of the Persian Gulf (Newton, 1955; Basson, 1979a; 1979b; 1992; Mshigeni and Dorgham, 1987; Dorgham 1990; De Clerck and Coppejans, 1994; 1996; Rizk et al., 1999; Al-Abdessalaam, 2007; John, 2005; 2012).

In the current study, we used a combination of morphology and molecular studies because of some complications in identifying the genus *Caulerpa* on the Iranian shores of the Persian Gulf and the Gulf of Oman, to make clear the species boundary of this genus in the area.

Material and methods

Sample collection

Samples of the *Caulerpa* genus were collected from different intertidal areas of the Persian Gulf and the Gulf of Oman on the southern coastlines of Iran. After cleaning all debris, the samples were washed with sterile seawater and dried in a paper towel, and then dried on herbarium sheets; a part of each sample was dried in plastic bags containing silica gel and stored at -20 °C. Voucher specimens were deposited algal herbarium of Agriculture and Natural Resources Research and Education Center of Hormozgan Province. Localities of collected samples are presented in Table 1.

Molecular studies

The silica gel dried samples were used for DNA extraction. The dried pieces of each specimen were subjected to total genomic DNA extraction using the Olsen CTAB modification method (Promega, Madison,

Table 1. Localities of collected samples at southern coastlines of Iran

Location	Station name	Longitude	Latitude
Jask	Jask coastal cape	57.764019	25.636320
	Saheli park	57.777129	25.640710
	Fajr seashore	56.232606	26.981241
Qeshm	Do koohak	56.210783	26.996001
	Governorship beach	56.264558	26.969403
	Govatr port	61.501707	25.165417
Chabahar	Ramin	60.735824	25.272164

WI, USA) or Qiagen Kit. The extracted DNA was used for PCR amplification of the chloroplastic gene, *tufA*, using the primers of stuff 5-TGAAACAGAAMAW-CGTCATTATGC-3 as forward and *tufAR* 5-CCTTCNCGAATMGCRAAWCGC-3 as revers (Fama et al., 2002). Polymerase chain reaction (PCR) was performed using a Multigene Thermal cycler TC9600-G (Lab Net International Inc, Edison, NJ, USA). The PCR reactions were performed using a PCR master mix of 13 µl consisting of 2.5 mM MgCl₂, 0.5 mm each primer, 0.2 mM each dNTP, 1.0 M Betaine, 0.5 units of Taq DNA polymerase purchased from Pishgam Biotech Company, Tehran, Iran (<http://www.pishgam.com>) and 0.5-1.0 µl of template DNA. The PCR program started with an initial denaturation at 96 °C for 5 minutes, followed by 40 cycles of denaturation (96 °C for 50 s.), primer annealing (48 °C for 1 min), and extension (72 °C for 2 min), followed by a final extension step at 72 °C for 4 min. PCR products were sent to Pishgam BiotechCompany (<http://www.pishgam.com>) for sequencing using the primers of the initial PCR reactions.

The obtained raw DNA sequences were edited using ChromasPro ver. 1.5 (Technelysium Pty Ltd, Queensland, Australia). The edited sequences were blasted in NCBI, and the sequences with higher similarities were accrued. The 35 sequences, including two sequences obtained current study and two sequences of *Caulerpella ambigua* (as outgroups), were aligned manually using BioEdit v.7.0.9.0 (Hall, 1999). They were rearranged with the Clustal X n.2.0.8 (Larkin et al., 2007). The best-fitted model for (1) maximum likelihood (ML) and (2) Bayesian (BI) analysis were selected using the results of KAKUSAN version3 (Tanabe, 2007) analyses. In the BI analyses, the Markov chain Monte Carlo method was used for 2000000 generations, and data sampling was done every 100 generations. The likelihood scores stabilized after 200,000 generations, and a 'burn-in' of 400,000 generations was used. To assess the level of variation in the provided sequences, the PAUP 4.0b.10 (Swofford, 2002) used to determine absolute distance and uncorrected pairwise genetic distances, excluding gaps and ambiguities. PAUP

version 4.0b.10 (Swofford, 2002) were used for maximum parsimony (MP) analyses which constructed trees using a heuristic search algorithm with 1000 random sequence additions, TBR branch swapping, using unordered and unweighted characters. The bootstrap values were generated using 1000 replicates.

Results

Morphological studies

Caulerpa selago (Turner) C. Agardh, 1817

Description: Thallus make a mat of dark to grass green in color on sandy or rocky substrates (Fig. 1A), consisting of creeping (true stolon with the rhizophorous branches) and erect axes Figure 1(B and

C). entirely covered with upwardly curved branchlets (ramuli) (Fig. 1D) at the lower parts of some erect axes, the branchlets collapsed and were conspicuously roughened by the stub bases of the discarded ramuli. Stolons dark green to pale in color, cylindrical, prostrate, creeping, and irregularly branched; erect axes are primarily simple, covered with closely imbricated, cylindrical, and acute ramuli. Vertical axes up to 30 cm high and prostrate stolons up to 2 mm diameter and can grow more than 1 m, ramuli simple, cylindrical, multi-seriate, alternate to irregularly arranged, upwardly curved, with mucronate apex, shrinking and wrinkled when dry and do not adhere to the herbarium sheets.

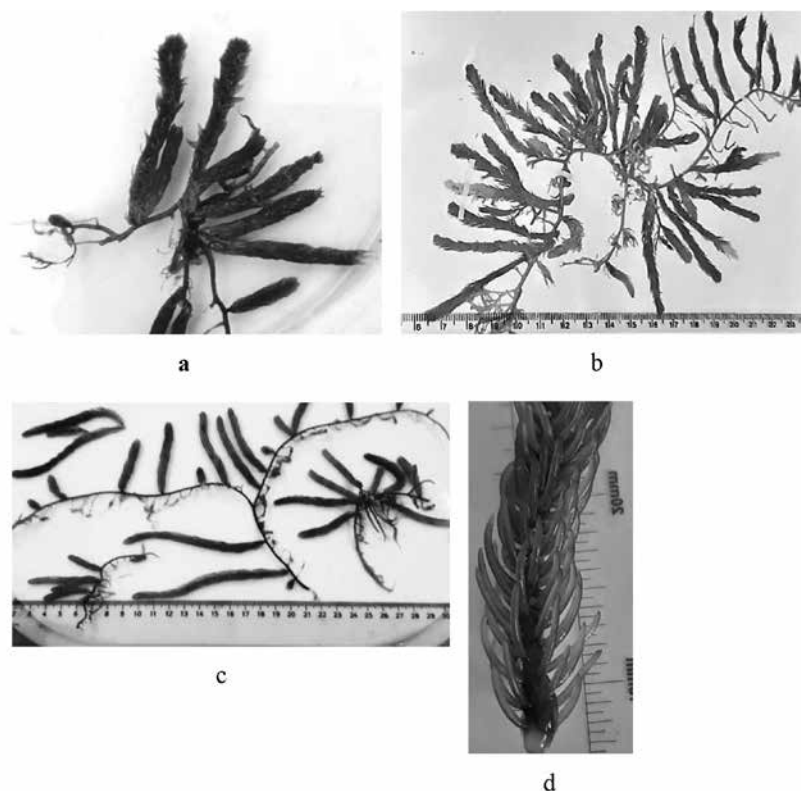


Fig. 1. *Caulerpa selago* collected from southern coastlines of Iran: (A) a mat of the species on sandy bottom, (B) dried herbarium specimens, (C) fresh samples of the specimens shows the creeping and erect branches and rhizoids (D) upwardly curved and mucronate form and size of ramulies on erect branches.

Molecular studies

The set of the sequences alignment contained 760 base pairs (bp), of which 578 characters are constant, 23 variable characters are parsimony-uninformative, and several parsimony-informative is 159. The aligned set consisted of 37 *tufA* sequences, including two new sequences generated in the current study and two sequences of *Caulerpella ambigua* as outgroups were subjected to three methods of phylogenetic analyzes. The results of the phylogenetic

analyses based on all three ways revealed that the two new generated sequences from Iranian coastlines of the Persian Gulf were clustered with a full bootstrap value in a clade, including a unique sequence from the samples of *C. selago*, which was submitted in GenBank from the Egyptian coast of the Red Sea (Fig. 2). This clade has a sister relationship with a branch including *C. amanuensis* and *C. cylindracea*, which biogeographically have distributed in subtropical and tropical areas.

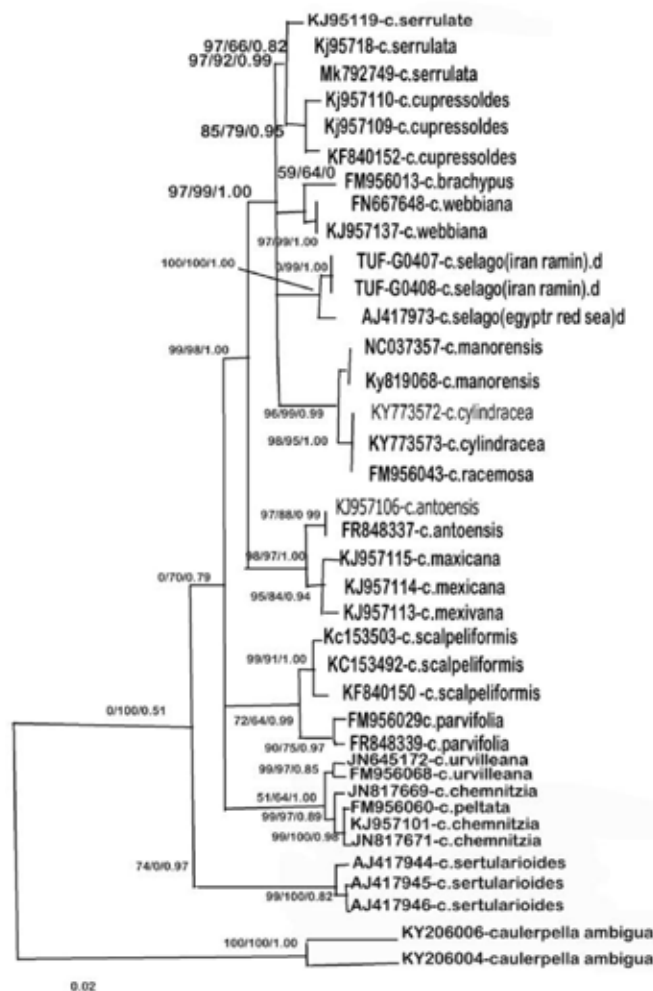


Fig. 2. The constructed ML tree based on 38 *tufA* sequences. Bootstraps and posterior probability support for each node are arranged as follow ML/MP/BI. The taxa in bold show the obtained sequences from Iranian specimens and *Caulerpa selago* from Red Sea.

Discussion

In this study, we aimed to do an accurate taxonomic analysis of *Caulerpa* members in the marine waters of the Persian Gulf and the Gulf of Oman on the southern coastlines of Iran. A part of the study focused on *Caulerpa selago* was presented in the current report. In the previous studies ten species including *C. fastigiata*, *C. manorensis*, *C. mexicana*, *C. peltata*, *C. racemosa* var. *macrophysa*, *C. sertularioides* f. *farlowii*, *C. sertularioides* f. *sertularioides*, *C. taxifolia*, *C. brachypus* and *C. scalpelliformis* have been reported from the area (Nizamuddin and Gessner, 1970; Sohrabipour and Rabiei, 1996; 1999a; 1999b; 2007; Sohrabipour et al., 2004; Kokabi and Yusefzadi, 2015).

In the images provided by Sohrabipour and Rabiei (1996, 1999a, 1999b), it seemed that the specimens of *C. selago* were reported as *C. sertularioides* f. *farlowii* (Weber Bosse) Børgesen (1907) based on Nizamuddin and Gessner (1970) report from Abu-Dabi, United Arab Emirate. These reports were repeated in published papers and checklists from the area (Sohrabipour and Rabiei, 2007; John and Al-Thani, 2014; Kokabi and Yusefzadi, 2015). All morphological evidence in the provided images in the mentioned studies is very similar to the morphological characteristics of the specimens studied in the current investigation. Based on the recent studies *C. sertularioides* f. *farlowii* (Weber Bosse) Børgesen was reported as a synonym with *C. sertularioides* f. *longipes* (J. Agardh) Collins in

the algaebase (Guiry and Guiry, 2021).

The morphological characters of the specimens studied in current research are in accordance with the diagnostic characters described for the *Fucus selago* by Turner (1808), which later changed to *Caulerpa selago* by Agardh (1817).

The measured sizes of the whole plant, erect branches, stolon diameter, ramuli size, and forms are very similar to the original description provided for the *Fucus selago* Turner (1808), which later were transferred to the *Caulerpa* genus by Agardh (1817). Genus *Caulerpa* is a relatively complicated group of the Caulerpaceae family. This complication has led to the nomenclature of too many species, subspecies, varieties, forms, and “ecads” in this genus. Most of these problems are caused by the morphological plasticity made by environmental conditions, which cause many mistakes in nomenclatures that, in turn, misunderstand the field of biodiversity, ecology, biochemical application, etc (Piazzi and Balata, 2009). Phylogenetic analysis as a modern biotechnological device helps overcome complicated issues in the taxonomic studies of different organisms. Many phylogenetic studies on the *Caulerpa* resolved some complex issues in this genus (Fama et al., 2002; Stam et al., 2006; Saunders and Kucera, 2010; Kazi et al., 2013; Sauvage et al., 2013; Draisma et al., 2014; Belton et al., 2014, 2015; Karthick et al., 2020; Zaw et al., 2020).

In the current study, by using a combination of molecular and phylogenetic analyses, we

resolved *C. selago* in the area which, based on morphological characters, has been reported *Caulerpa sertularioides* var. *farlowi* from Abu Dhabi, UAE (Nizamuddin and Gessner, 1970; John, 2005; 2012; John and Al-Thani, 2014); Bahrain (Silva et al., 1996; Rizk et al., 1999), Saudi Arabia (De Clerck and Coppejans, 1994; 1996; Silva et al., 1996), Iran (Sohrabipour and Rabiei, 1999a; 1999b; Sohrabipour et al., 2004; Rabiei et al., 2005; Sohrabipour and Rabiei, 2007, Shams and GhaedAmini, 2017). It should be considered that the record by Nizamuddin and Gessner (1970) was the first mention of the variety from Abu Dhabi at the coastlines of the Persian Gulf. The achieved results showed the presence of *C. selago* species on the southern coasts of Iran. Sequences of Iranian samples well matched with the only one sequence of *C. selago* specimens submitted in genBank (NCBI) from the Egyptian coast of the Red Sea. These results also showed that the *tufA* gene could help distinguish the members of this genus at the species level and overcome the morphological complication of the genus members.

C. selago is the most common *Caulerpa* species on the southern coastlines of Iran and can grow in extended mats on protected sandy-covered rocks and sandy bottoms in the winter and spring seasons. The results revealed more studies need to do on the taxonomic, ecological, biochemical aspects, and commercial application of the species in this genus.

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